1934

The relation of the size of the infective dose to number of oocysts eliminated, duration of infection and immunity in Eimeria miyairii infections in the white rat

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THE RELATION OF THE SIZE OF THE INFECTIVE DOSE TO NUMBER OF OOCYSTS ELIMINATED, DURATION OF INFECTION AND IMMUNITY IN EIMERIA MIYAIRII INFECTIONS IN THE WHITE RAT

BY

PHOEBE REBECCA HALL

A Thesis submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major subject Zoology

Approved

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I. ACKNOWLEDGMENTS

The writer wishes to take this opportunity to express her deep appreciation and sincere gratitude to members of the Department of Zoology and Entomology of Iowa State College by whose friendly interest and kind alleviations the task of the present study has been materially lightened.

Thanks are extended especially to Dr. Elery R. Becker for his assistance in outlining the problem, for his many valuable constructive suggestions, and particularly for his constant readiness to offer enthusiastic direction and encouragement throughout the progress of the investigation, and to Professor J. E. Guthrie for his zeal and alacrity in correctively reading the manuscript.

II. INTRODUCTION

The original description of the coccidian parasite Eimeria miyairii was published in Japanese by Ohira in 1912. The endogenous cycle occurs in the small intestine, caecum, and colon of the rat and requires a minimum of approximately seven days for its completion. At the end of this prepatent period, oocysts appear in the fecal pellets. It was one of the objects of the present investigation to determine whether the size of the infective dose bore any relationship to the length of the period of elimination of oocysts. The oocysts are, in general, slightly egg-shaped and measure on an average eighteen microns in breadth and twenty-three microns in length.

Perard (9) first noted that infection by this organism may produce a diarrhea in young culture rats, leading in many cases to death. He did not make any study, however, of the approximate number of sporulated oocysts required to produce a fatal infection. Since loss of appetite and emaciation are recognized symptoms in severe coccidiosis, weight changes would be an important measure of physiological disturbance produced by an infection. In an effort, therefore, to determine the relative morbidity of the infections the daily gain or loss in weight was recorded.

The number of oocysts eliminated following a series of five infective doses of 1500 oocysts each was reported in

1932 by Becker and Hall as falling between 14,100,000 and 169,220,000. Up to this time, however, no study has been made to determine the yield from a single dose of a known number of sporulated oocysts. It was felt that information concerning the normal number of oocysts produced by any known number of parasites would be of value in the study of immune effects produced during an infection, and also would determine the optimum dose for experimental purposes with the rat as the host. It was with these motives in view that the author has attempted to determine the relationship which exists between the numbers of sporulated oocysts fed to an animal and the number of parasites passed in the feces of the infected animal during the period of oocyst elimination.

It was reported by Becker and Hall (1932) that the feeding of 1500 oocysts on each of five consecutive days produced a total immunity. Whether this entire number was required for the production of this immunity was not known at that time. It was believed, however, that smaller doses would produce a partial immunization which would prevent a subsequent heavy reinfection. Should this be the case for all coccidial infections, an experimental pattern could perhaps be worked out which by its practical application would prevent losses of domesticated animals due to severe coccidiosis, and still not produce any significant injury to the host.

Briefly stated, the present study, therefore, was designed to make the following determinations: (1) the number of oocysts eliminated following the feeding of various sized doses of viable sporulated oocysts; (2) whether the length of time during which oocysts were passed in the feces varied with the size of the infective dose; (3) the effect of different doses on the mean daily weights; (4) the relative amount of immunity produced by small infective doses and the minimum number of sporulated oocysts required to produce total immunity; and (5) the pathological effects of different sized infective doses of the sporulated oocysts.

III. METHODS

Rats used in these experiments were for the greater part Wistar A rats, and were offspring of closely related parents. By using rats of such parentage, all experimental animals had a similar genetical constitution and consequently differences in yields due to hereditary differences in susceptibility of the host were avoided as much as possible. It was noted that even then there appeared litters which as a group outyielded other litters which had received a duplicate treatment. From the time of birth until approximately four weeks later, each litter of young rats was kept singly in a breeding cage, the bottom of which was kept covered with a carpet of dry shavings.

The young rats, after being weaned, were fed a modification of the Steenbock growth ration, green foods at intervals of three or four days, and milk and water ad libitum. The ration used was as follows:

Yellow corn meal	76.0 lbs.
Linseed oil meal	16.0 "
Commercial casein	5.0 "
Ground alfalfa	2.0 "
NaCl	0.5 "
$CaCO_3$	0.5 "
Dried buttermilk	12.0 "

At the age of five weeks, the rats were put on experiment. At this time each animal was placed individually into a specially made cage of three mesh hardware cloth fitted over a 9" x 12" aluminum or enamel pan. The rats were weighed daily after being put on the experiment. The diet, thereafter, consisted of only the grain mixture and water. Previous to the time of the experimental feeding of oocysts, none of the rats had an infection. Until then, all young growing rats were examined at regular intervals of three or four days, and had there been accidental infections, such would have been detected.

The parasite used in the experiment was a selected strain of Eimeria miyairii. This strain was selected and inbred in the following manner. With a micropipette a single sporulated oocyst was secured from a field under low (10X) power of the microscope. Then by placing the pipette into the mouth of a partially anesthetised rat this oocyst was administered to its host. The oocysts which developed from this one were collected in a two percent solution of potassium dichromate, and from this culture another single oocyst was isolated and similarly administered to another rat. This process was repeated until the parasite had been inbred for the third generation. It was believed at this time that whatever oocysts might be selected by chance from this highly inbred strain would be representative of a fairly homogeneous

population.

From thirty-three attempts to infect rats with a single parasite, only twenty-two trials proved to be successful. From these figures it was concluded that only two-thirds of the number of oocysts given to any one rat succeeded in completing the sexual phase of their life history in the intestine. This assumption was thereafter respected in the calibration of all cultures used for infective purposes; for example, an infective dose of ten oocysts consisted of actually fifteen.

In the determination of the yield of oocysts from a given number of parasites, the infective doses contained the following number of organisms: one, four, ten, fifty, one hundred, fifteen hundred, fifteen thousand, and one million five hundred thousand. In isolating a single dose of one, four, or ten oocysts, the oocysts were selected singly from a field under low power of the microscope. In preparing these small doses, the actual selection consisted of one, six, and fifteen oocysts respectively. In giving doses of fifty oocysts or more, the dilution method was used in measuring the dose. In each of the latter cases, the culture was so calibrated that the desired number of parasites was suspended in one cubic centimeter of liquid.

The rats were etherized and given the one cubic centimeter of the suspension containing the oocysts from a syringe through a catheter. For each group of rats infected with the different sized doses, a number of rats of the same litter were kept for controls.

On the sixth day after the date of infection the pans under the experimental animals were thoroughly cleaned and to each was added three hundred cubic centimeters disinfectant. The disinfectant used varied from a twenty-five hundredths percent to a five-tenths percent solution of "Kreso", the approximate chemical composition of which is Cresol, two and five-tenths percent; soap (dry), twenty-three percent; and inert ingredients, seventy-four and five-tenths percent.

The fecal pellets were allowed to collect in the pans until elimination of oocysts ceased. The pellets were then broken up by mashing with a miniature mallet consisting of a solid one inch rubber stopper into one end of which was inserted a glass stirring rod one-fourth inch in diameter and five inches in length. The suspension of fecal matter in the disinfectant was transferred from the collecting pan to a heavy glass container in which it was thoroughly homogenized by an electric mixer. The mixture was diluted with water, the exact dilution depending upon the amount of solids in feces collected. After the material was again thoroughly agitated, the larger solid particles were removed by strain-

ing a small sample through wire screens. This process was done in a fashion which averted a probable straining out of oocysts by solid particles accumulated on the filter. Screens through which the material was strained were of two sizes; namely, sixteen and twenty-four mesh respectively. Following another thorough mixing a small amount of the suspension was immediately transferred to a haemocytometer three-tenths millimeter square and one-tenth millimeter deep. For determining the yield from a single parasite, the counting chamber, or haemocytometer, was filled twenty times. For the larger doses, however, only eight or ten counts were made, the number of counts depending on the extent of variation of the first eight. Then from the known number of oocysts in either eighteen, nine, or seven and two-tenths cubic millimeters, the total yield was calculated by multiplying the number of parasites counted by the ratio of the total volume of diluted material to the volume containing the oocysts counted.

To determine whether the rats which had been infected with a given known number of oocysts were either partially or entirely immunized, the previously infected experimental animals together with the controls were given fifteen hundred oocysts on each of five consecutive days. This is the dose previously reported by Becker and Hall as developing total

immunity. As a precaution against physiological interferences between the two infections the attempts at reinfection were not made until the cessation of oocysts produced by the first infection. The same treatment was given to rats during the second infection as during the first and the previously discussed technique employed in making determinations of oocyst yields was again used. The yield of oocysts from previously infected rats compared with that of the controls was taken as an index to the amount of immunity produced by the various sized infective doses.

IV. RESULTS

1. Yield of oocysts from single infective doses of various sizes.

For the study of the number of oocysts which the host would eliminate when inoculated with a dose of a known number of sporulated oocysts, there was carried out a series of experiments in which selected groups of rats were designated to receive different numbers of infective organisms, each rat in a group receiving the same number. The numbers of sporulated oocysts in the test dosages for each group were one, four, ten, fifty, one hundred, fifteen hundred, fifteen thousand, and one million five hundred thousand, respectively. Counts of the oocysts eliminated by the individual rats on the experiment showed that the greater the infective dose of parasites the greater was the yield of oocysts, but the ratio was not by any means constant. Statistically, the coefficient of correlation between the number of sporulated oocysts in the infective doses and number of oocysts yielded from them (i.e., the mean yield for each test group receiving a particular sized inoculation) was .3184. The regression of this value is $A=940X + 133 \times (10)^4$, where A represents the yield from the number of oocysts in the infective dose X. From this regression could be roughly estimated the approximate

number of oocysts that would be produced by any sized infective dose.

The counts of the oocyst yielded by the individual rats on the experiment are listed in Table I.

TABLE I

Oocysts produced by individual experimental hosts with infective doses of different sizes

1 oocyst		4 oocysts		10 oocyst		50 oocysts		100 oocysts		1500 oocysts		15,000 oocysts	
No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4
1	5.56	71	730	29	1322	250	4861	109	16861	146	25860	187	46083
2	6.66	73	550	30	2222	251	9225	110	34176	147	17751	188	44892
3	5.44	77	1167	31	1299	252	9570	111	20264	148	21747	189	36178
4	6.66	78	1144	33	3544	253	7175	112	24558	149	23110	191	49512
5	6.83	79	778	38	3354	254	7650	116	14924	150	25950	192	37528
6	5.83	80	950	39	3777	259	3133	117	19892	156	39823	281	24667
7	5.26	81	1017	42	1676	260	9917	118	16100	157	34267	284	28917
8	5.54	87	412	43	2136	261	3911	119	7807	158	34267		
9	6.27	88	533	47	1033	269	7126	120	10286	159	39998		
10	5.83	89	631	48	1167	270	11288	125	10937	160	27067		
11	7.32	90	770	49	1239	271	5163	126	10704	161	26795		
12	7.15	91	1262	50	2655	272	8080	127	10000				
13	6.75	92	1111	51	1706	277	10375	128	8305				
14	6.17	95	806	52	2117	278	14700	129	11413				
15	7.25	96	676	53	2011	279	9400						
16	7.08	97	888			280	10200						
17	6.39	103	1138										
18	5.17	104	1280										
19	7.17	105	746										
20	5.83												
23	5.50												
24	5.75												
M	6.24	M	873	M	2084	M	8235	M	15445	M	28830	M	38254

TABLE I

ual experimental hosts with infective doses
of different sizes

oocysts	100 oocysts		1500 oocysts		15,000 oocysts		1,500,000 oocysts		
No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4	No.	Yield /10^4
50	4861	109	16861	146	25860	187	46083	228	155000
51	9225	110	34176	147	17751	188	44892		
52	9570	111	20264	148	21747	189	36178		
53	7175	112	24558	149	23110	191	49512		
54	7650	116	14924	150	25950	192	37528		
59	3133	117	19892	156	39823	281	24667		
60	9917	118	16100	157	34267	284	28917		
61	3911	119	7807	158	34267				
69	7126	120	10286	159	39998				
70	11288	125	10937	160	27067				
71	5163	126	10704	161	26795				
72	8080	127	10000						
77	10375	128	8305						
78	14700	129	11413						
79	9400								
80	10200								
I	8235	M	15445	M	28830	M	38254	M	155000

In Table II are recorded the mean yields from the different sized infective doses, and the number of oocysts produced per oocyst fed in the various infections. When only one oocyst was fed there was a mean yield of approximately 62,000, while following an infective dose of four viable sporulated oocysts 2,182,500 oocysts per oocyst fed were produced. Why the yield per oocyst given for four oocysts should be thirty-five times that of the one oocyst infection is at present unexplainable. It might perhaps be due to increased chances for the union of the microgametes and the macrogametes in the case of the larger infective dose. It can be readily seen that an increase in number of parasites fed would cause the liberation of merozoites in larger quantities which in turn would develop into more gamete-producing cells. The larger the number of gametes, the greater their proximity, and consequently the greater the likelihood of the microgametes encountering the macrogametes. This, however, does not explain why the oocyst production per oocyst fed should again gradually decrease from 2,084,000 when ten oocysts were fed to 1,000 when fifteen hundred thousand were fed. The latter may be an immunity phenomenon. The nature of the immunity produced nor the exact time required for its production is not known, but here again the marked comparative decrease in yield per oocyst in the case of the higher dosage might perhaps be due to a partial immunization by the larger

numbers of merozoites which prevents the development of a proportionate number of gametocytes, and hence gametes. There remains also the possibility that there is during the infection a temporary depletion of invadable epithelial cells. It seems, however, that if this were the case, the total oocyst yield would be constant after a maximum was once reached.

TABLE II.

Number of oocysts produced by different size infective doses

Number of animals	Number of oocysts in infective dose	Mean yield/$(10)^4$	Standard deviation/$(10)^4$	Oocysts produced per oocyst fed/$(10)^4$
22	1	6.24 ± .029	.21	6.24
19	4	873 ± 40	260	218.25
15	10	2084 ± 155	892	208.40
16	50	8235 ± 502	2977	164.70
14	100	15445 ± 1377	7360	154.45
11	1500	28830 ± 1323	6472	19.22
7	15000	38254 ± 2336	9147	2.61
1	1500000	155,000		.10

2. Duration of Infection.

TABLE III.

Effect of size of dose on duration of infection

Number of oocysts in infective dose	Number of rats on experiment	Prepatent period			Patent period			
		7 days	8 days	Mean	3 days	4 days	5 days	Mean
1	22	8	14	7.6	2	12	8	4.2
4	19	9	10	7.5	1	12	6	4.2
10	15	7	8	7.5	1	8	6	4.3
50	16	9	7	7.4		8	8	4.5
100	14	6	8	7.5	1	8	5	4.3
1500	11	7	4	7.3		3	8	4.7
15000*	7	5	2	7.2		1	6	4.8
1500000*	1	1		7.0			1	5.0

*The data given here apply to only rats which survived throughout the infection.

It was detected by daily fecal examination that all rats on the various experiments began to eliminate oocysts either on the seventh or the eighth day after infection. According to Andrews (1) this time required for the endogenous cycle to become completed is the prepatent period. It will be seen from Table III that the first appearance of oocysts was in the majority of cases on the seventh day. Even though oocysts were not found on the seventh day, all fecal matter after the sixth day was saved. This precaution was taken for fear there were a few oocysts eliminated by all rats on the seventh day, but that due to random sampling the fecal smears examined did not reveal their presence. In no case was the oocyst yield at its maximum intensity before the ninth or tenth day after the administering of the viable oocysts. Table III also gives the duration of the period of oocyst elimination in days. It will be there noted that this period varied from three to five days inclusive. The data show that the mean patent period (so-called by Andrews) varies from 4.2 days following the feeding of small infective doses to 5 days following an infective dose of 1,500,000 oocysts. The author questions whether there is actually an increase in the length of the period of oocyst elimination corresponding to the increase in number of oocysts fed, or whether this superficial difference is due merely to the oocysts being

present in larger numbers and consequently an increase in the chances for their being seen in the sample of feces examined, sooner after elimination begins and for a longer period after the time of maximum yield has been reached.

3. Immunizing effects of different sized doses.

As previously stated, it was believed that the standard immunizing dose as adopted by Becker and Hall was larger than was actually needed to produce a total immunity. It was suggested by Johnson in 1927 (8) and again by Tyzzer in 1929 (10) that a small dose prevented a heavy subsequent re-infection, but no quantitative study of immunity produced by small infective doses was made. In the effort to determine the minimum dose which would cause an appreciable loss in susceptibility of the host, there is evidence that an infection as small as that produced by four oocysts will produce a degree of immunity worthy of note. The yields from the multiple infections following a single infection of 4 oocysts ranged from 680 x $(10)^4$ to 2,292 x $(10)^4$, while the yield from the controls for that same group ranged from 2,292 x $(10)^4$ to 26,187 x $(10)^4$.

Testing this difference by the pooled sum of squares method, recommended by Fisher (7) for small samples, gives a value of 3.77 for t. Fisher states that a value of only

2.724 for t is sufficient to denote differences which are highly significant; i.e., this difference would be expected to be found in at least ninety-nine percent of all similar experiments.

These experiments were repeated using ten, fifty, one hundred, fifteen hundred, and fifteen thousand oocysts respectively, the significance of the results of which were statistically tested and summarized in Table IV.

TABLE IV

Significance of susceptibility lost due to previous infection

Oocysts in first infection	No.	Mean yield x $(10)^4$	Standard deviation x $(10)^4$	Lowest yield x $(10)^4$	Highest yield x $(10)^4$	Value of t	Value of t above which differences are highly significant
4	19	7,840 ± 1055	6805	680	28368		
						3.77	2.724
Controls	18	16,525 ± 1172	7202	2292	26187		
10	15	4,069 ± 680	3902	221	11259		
						4.988	2.763
Controls	15	17,818 ± 1592	9135	5618	33913		
50	15	1,447 ± 108	625	425	8300		
						4.618	2.763
Controls	14	22,379 ± 3035	9200	7978	36650		
100	14	223 ± 26	144	14	569		
						4.34	2.807
Controls	11	20,500 ± 3817	18730	5597	58944		
1500	11	.636 ± .410	2013	0	7		
						7.157	2.845
Controls	10	11,259 ± 1020	4780	5069	20847		
15000	7		-	-	-	-	-
Controls	14	10,128 ± 685	3210	5221	18100		

Table V shows that there is conclusive evidence that a single infection produces an appreciable amount of resistance in the body of the host. The question arises as to just how much susceptibility has been lost by the host due to a known number of parasites, and from the standpoint of the host what is the optimum dose for producing sufficient immunity for protection against subsequent reinfection.

In order to get the loss of susceptibility due to the different sized doses in comparable terms, the author has attempted to estimate the percent of total immunity acquired during the first infection by assuming the controls for each group to be 100 percent susceptible. The percent of immunity acquired was then figured by obtaining the following ratio:

$$\frac{M \times N \times 100}{Mc \times Nc}$$

Where M and N are the mean of yield and number of experimental animals, and Mc and Nc are the mean of yield and number of controls, respectively.

Figuring the percentages in this way, infective doses as small as 4 oocysts were found to produce as much as 53.02 percent immunity, while a single dose of 1,500 oocysts caused a loss of 99.995 percent of total susceptibility. None of the infections due to 1,500 oocysts or less seem to produce any apparent clinical symptoms.

Table V gives the fraction of total immunity gained by different sized doses. It is of interest to note that a dose of 1,500 oocysts produced 99.995 percent total immunity. There were in this group eleven experimental animals, ten of which were completely immunized after the single infective dose of 1,500 oocysts.

TABLE V.

Effect of previous infection on oocyst yield from standard immunizing dose.

Number of oocysts in previous infective dose	Mean yield from previous infection	Oocysts produced per oocyst fed to experimentals	Oocysts produced per oocyst fed controls	Percent of total immunity produced by first infection
4	$873 \times (10)^4$	10,452	22,033	53.02
10	$2,084 \times (10)^4$	5,429	23,757	77.15
50	$8,235 \times (10)^4$	1,930	20,000	90.35
100	$15,445 \times (10)^4$	293	27,300	98.03
1500	$28,830 \times (10)^4$	8	15,012	99.995
15000	$38,254 \times (10)^4$	0		100.00

4. Weight changes, pathological effects and lethal dosage.

No apparent clinical symptoms were evident in rats receiving an infective dose of one or four oocysts. In an infection resulting from ten to fifteen hundred oocysts, the most noticeable symptoms were general sluggishness and loss of responsiveness to raps on the cage on about the seventh and eighth days after the date of infection. Not until the single infective dose was increased to 15,000, did the experimental animals gain weight at an apparently significantly less rapid rate than did normal rats during the same period.

In the lighter infections there were days on which there was no appreciable gain, but corresponding weight changes were usually observed in the control animals on the same days. Rats which survived following the administering of 15,000 oocysts showed a mean decrease in weight of -.2, -7.1, and -2.3 grams, on the seventh, eighth, and ninth days respectively. This observation is of still greater evidence when it is taken into consideration that forty-six percent of animals infected with this sized dose died on the eighth day after the date of infection and that the animals here considered represent the more endurant group of the rats which were infected.

Presented in tabular form in Tables VI and VII is a synopsis of the mean daily oscillations in weight of both the experimental and control animals.

TABLE VI

Effect of different size infective dose on mean daily gain in weight in grams.

Day after infection	Number of oocysts in infective dose															
	1		4		10		50		100		1500		15000		1500000	
	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.
1	5.8		5.5	6.5	4.2	3.0	4.5	5.3	1.4	4.8	3.0	4.7	3.0	2.4	1	3
2	4.5		1.5	1.1	5.5	3.6	3.6	4.3	5.8	3.8	2.8	2.5	3.3	2.9	0	16
3	3.0		2.8	6.1	3.8	4.7	3.6	4.4	4.0	5.3	8.0	6.5	4.6	3.0	2	7
4	3.1		7.8	2.7	3.8	4.5	5.2	3.3	4.7	2.7	2.8	4.0	.8	3.2	7	4
5	3.6		6.4	5.1	6.2	4.8	4.4	3.3	4.8	6.1	-1.2	3.3	5.6	3.0	4	5
6	3.3		3.6	7.6	4.1	3.4	2.9	2.0	3.2	4.0	11.7	6.0	1.8	5.1	3	10
7	2.4		5.0	4.7	2.6	4.3	2.6	4.5	2.6	4.8	-.7	5.5	-.2	-2.0	-2	-2
8	3.8		3.4	1.2	5.7	4.2	1.2	3.5	1.4	3.1	-3.8	-.1	-7.1	2.2	-2	7
9	2.4		3.7	5.9	5.2	4.5	5.2	3.3	3.5	4.0	3.8	4.6	-2.3	1.6	12	2
10	3.0		4.7	2.6	4.2	4.0	5.6	4.5	2.8	5.1	4.2	5.4	3.0	2.9	14	5
11	2.0		2.7	.6	4.0	4.3	4.6	3.5	5.2	.9	6.1	3.6	2.7	2.7	2	2
12	1.8		6.0	6.6	3.0	4.0	3.2	2.4	3.7	5.8	6.5	1.5	2.5	3.8	2	2
Initial weight	100		79	80	79	79	83	75	87	83	85	82	77	70		
Total gain during infection	39		53	51	52	44	47	44	43	50	43	47	18	31		
Percent gain over initial weight	39		67	63	66	62	56	57	49	60	50	58	23	44		

It will be seen from the percentage gains listed near the end of Table VI that the rats receiving 100 oocysts or more gained an apparently significant smaller percentage of their initial weight than did their respective controls. There was not, however, sufficient data obtained from the present investigation to justify a prediction of this nature since the weight of the rat has such a high degree of normal fluctation. A casual glance at the weight of the controls will indicate that there are at times rather astounding day to day variations under what could be termed normal physiological conditions. The investigator believes, therefore, that to place too much emphasis on the gain or loss of weight due to the infection would be misleading since some of the differences might chance to be due merely to physiological coincidents rather than to pathological conditions.

Following an infection with a dose as large as 15,000 oocysts, however, there seems to be during the infection a percent gain over the initial weight which is significantly smaller than that of the controls during the same period. The experimental animals in this group weighed an average of 77 grams at the beginning of the infection and gained during the experiment twenty-three percent of their initial weight, while the controls weighed to begin with only 70 grams and before the end of the experiment had increased in weight forty-four percent. This difference was most noticeable during the first nine days of the infection.

TABLE VII

Effect of previous infection on mean daily gain in weight in grams.

Day after infection	Number of oocysts in previous infective dose															
	1		4		10		50		100		1500		15000		1500000	
	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.
1			1.9	4.9	3.1	1.5	-.4	2.1	4.0	3.6	6.1	8.6	1.7	6.2		
2			3.1	2.9	5.0	5.6	3.6	2.3	.6	1.2	2.8	.3	2.8	2.2		
3			0	1.5	4.6	4.4	2.5	2.3	6.5	6.4	8.2	6.4	1.7	1.4		
4			4.0	1.8	3.5	3.2	-.6	-.3	3.9	3.2	2.8	.7	3.0	4.5		
5			3.2	5.5	4.8	4.0	2.6	3.0	2.1	-.7	-.9	-1.6	2.0	2.7		
6			-.9	2.7	3.1	2.4	1.6	1.4	-.3	3.6	1.6	5.1	.4	1.3		
7			6.5	1.6	4.1	5.6	1.5	-1.3	2.5	1.6	6.8	3.2	6.3	.6		
8			.8	2.3	4.0	2.3	2.5	.4	.6	-.6	2.2	-1.2	4.5	-2.0		
9			2.9	.9	4.3	2.8	2.0	0.0	4.2	4.7	3.7	5.6	3.4	.2		
10			4.1	2.1	3.6	5.0	7.0	5.6	5.0	1.0	3.4	3.7	3.8	3.7		
11			3.4	2.0	1.8	1.6	4.4	8.6	.8	2.4	7.2	5.3	2.6	3.7		
12			1.7	1.7	5.3	5.3	5.1	3.2	4.5	5.1	1.5	4.3	3.1	2.1		
13			5.5	2.6	1.8	2.0	3.3	2.8	1.5	8.1	5.2	4.4	2.0	4.1		
14			2.6	2.1	3.9	4.6	6.0	5.3	1.1	2.2	2.8	3.0	2.9	1.6		
Initial weight			136	135	127	120	136	128	137	140	130	130	98	101		
Total gain during infection			39	35	53	51	40	35	37	42	53	48	37	34		
Percent gain over initial weight			19	26	42	42	22	27	27	30	41	37	37	34		

All rats reported in Table VII received a series of five infections, each consisting of 1,500 viable oocysts. The giving of the five infective doses was equally distributed over a period of five days. The experimental animals had received approximately two weeks before the date of this multiple infection, a single infective dose of viable oocysts.

The gain of weight does not indicate any effect due to the previous infection. There is in most cases a difference (either positive or negative) between the percent gain of initial weight in experimental and in control animals. This difference, however, is believed by the author to be no greater than would be expected from random samples of the same population.

It was found that by feeding 15,000 oocysts there was a forty-six percent mortality on the eighth day of the infection, whereas with a larger dose of 1,500,000 oocysts, ninety-two percent of the fourteen rats died within thirty-six hours after being infected. All rats in either case developed a severe diarrhea before death, and a post mortem examination showed the small intestine to be markedly haemorrhagic.

V. SUMMARY AND CONCLUSIONS

1. The mean yield of oocysts of Eimeria miyairii from single infective doses of different sizes were as follows:

1	oocyst	62,000
4	"	8,730,000
10	"	20,840,000
50	"	82,350,000
100	"	154,450,000
1500	"	288,300,000
15000	"	382,540,000
1500000	"	1,550,000,000

2. The prepatent period for a single infective dose ranging from one to 1,500,000 sporulated oocysts is approximately seven days. The patent period for the same varies from three to five days, and in the majority of cases is either four or five days. Its length seems to be independent of the size of the infective dose.

3. Different infective doses are in no way reliably indicative of a general predictable gain or loss in weight during the infection. There is naturally a big variation in the daily gain of weight, but this variation is not, in general affected by the coccidial infection of any sized infective dose. Single infective doses of 15,000 oocysts, however, cause an increse in weight during the infection which is significantly less than that of the controls during the same period of

time. The significance of this difference is most manifest during the first nine days of the infection.

4. Single infective doses as small as four viable sporulated oocysts will cause a rat to lose approximately fifty percent of its natural susceptibility; ten oocysts, approximately seventy-five percent; fifty oocysts, approximately ninety percent; one hundred oocysts, approximately ninety-eight percent; and fifteen hundred, approximately one hundred percent.

5. Single infective doses of 15,000 viable oocysts prove fatal on the eighth day of the infection in approximately fifty percent of the cases, while with a still larger dose of 1,500,000 sporulated oocysts there is approximately a ninety-five percent mortality within thirty-six hours after giving the parasites.

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